3RD

INTERNATIONAL VERTICILLIUM SYMPOSIUM

Istituto di Patologia Vegetale
University of Bari, Italy
1981

CONTENT

FOREWORD

Besides having a wide polyphagy, certain plant pathogenic fungi such as *Verticillium* spp. are also characterized by a high environmental adaptability. They are thus common to many geographical areas under extremely variable climatic conditions and are therefore of widespread general importance. The complexity of Verticillium diseases and the great economic importance of yield loss caused by them have brought on the interest of an international group of scientists who spontaneously promote meetings to compare and discuss the results of studies carried out all over the world on Verticillium wilt in numerous species of cultivated plants. The excellent results experienced in the two previous Symposia held at Wye (U. K.) and Berkeley (Ca., U.S.A.) encouraged us to organize the 3rd International Verticillium Symposium at the Istituto di Patologia vegetale of the University of Bari, Italy.

Achievements in research presented in this Symposium and obtained in the past five years on epidemiology, ecology, genetics of *Verticillium* spp., pathogen-disease physiology, genetics of host resistance, and chemical control will certainly improve our knowledge of Verticillium diseases and their control, thus contributing to a greater and more rewarding efficiency in the work of man in agriculture. With the certainty of this goal in mind, I would like to take this opportunity to extend to all participants in this Symposium my best wishes for a pleasant and fruitful stay in Italy.

Matteo Cirulli

Acknowledgements

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PROGRAM

3rd INTERNATIONAL VERTICILLIUM SYMPOSIUM
Istituto di Patologia Vegetale - University of Bari - Italy
August 25-28, 1981

PROGRAM

Monday, August 24

Afternoon:

- Arrival in Bari
18.00 - Registration and refreshments at Jolly Hotel

Tuesday, August 25

Morning:

8.30 - Registration (late).

9.30 - Opening address.

Session I: Impact of Verticillium diseases in agriculture
Chairman: J. KRIKUN

10.00 - G. F. PEGG (U.K.): The impact of Verticillium diseases in agriculture.

10.30 - D. L. EBBELS (U.K.): Administrative measures for control of progressive Verticillium wilt of hops in England.

10.45 - H. MOSHIR-ABADI (Iran): *Verticillium dahliae* Kleb. in Iran.

11.00 - Coffea & Tea.

11.30 - L. ZAMBITO and O. APONTE (Venezuela): Occurrence of *Verticillium* spp. in Venezuela.

11.45 - G. EVANS, A. FRANCIS and J. KALDOR (Australia): Assessment of methods for measuring losses caused by *Verticillium dahliae* in cotton.

12.00 - A. M. EL ZARKA (Egypt): Occurrence and distribution of Verticillium wilt of sunflower in Egypt.

12.15 - A. FRANCIS, G. EVANS and J. KALDOR (Australia): Quantitative measurement of losses caused by *Verticillium dahliae* in cotton.

13.00 - Lunch.

Session II: Epidemiology and ecology
Chairman : W. E. SACKTON

14.30 - J. E. DE VAY and G. S. PULLMAN (U.S.A.): Epidemiology and ecology of diseases caused by *Verticillium* species. With emphasis on Verticillium wilt of cotton.

15.00 - G. S. MUROMTSEV, T. M. LAGUTINA, G. A. GLOBUS, O. K. STRUNNIKOVA and I. I. CHERNIAVEVA (U.S.S.R.): A study of the *Verticillium dahliae* Kleb. ecology in soil with the use of membrane chamber techniques.

15.15 - T. WATANABE (Japan): A new variety of *Verticillium sphaero-sporum,* and endoparasite of nematodes and antagonist of soilborne plant pathogens.

15.30 - C. C. THANASSOULOPOULOS, D. A. BIRIS and E. C. TJAMOS (Greece): Occurrence of Verticillium wilt in weeds in olive orchards.

15.45 - E. C. TJAMOS (Greece): Race 2 and defoliating T-1 strain of *Verticillium dahliae* in Greece and other mediterranean countries.

16.00 - S. VISSER and M. J. HATTINGH (Republic of South Africa): Relationship of inoculum of *Vertificillium dahliae* to disease in tomato plants.

16.15 - Coffea & Tea.

16.45 - N. J. WHITNEY and K. S. HUNG (Canada): Studies on germination of spores of *Verticillium albo-atrum.*

17.00 - D. P. GUPTA (India): Variations in strains and species of *Verticillium.*

17.15 - J. COOSEMANS (Belgium): Estimation of the distribution and pathogenicity of *Verticillium* populations in soils using selective baitplants.

17.30 - H. A. MELOUK, and D. F. WADSWORTH (U.S.A.): Effect of plant age on the susceptibility of the peanut cv. Tamnut to Verticillium wilt.

17.45 - V. ZINKERNAGEL (West Germany): Some aspects concerning the susceptibility and tolerance of hop varieties agaist Verticillium wilt.

18.00 - F. S. SEDUN and W. E. SACKSTON (Canada): The effect of daylength on Verticillium wilt of sunflower.

Wednesday, August 26

Morning:

Session III: Genetics of *Verticillium* spp.
Chairman : J. E. PUHALLA

9.00 - A. C. HASTIE and J. B. HEALE (U.K.): The genetics of *Verticillium*.

9.30 - M. A. TYPAS (Greece): A cytoplasmic system of inheritance affecting the variability and differentiation of *Verticillium*.

9.45 - J. B. HEALE, J. M. CLARKSON and G. P. HARRIS (U.K.): Strain specialisation, genetic compatibility and colonisation studies in Verticillium wilts of lucerne and hop.

10.00 - C. BOISSON and H. LAHLOU (Morocco): Intraclonal morphological variations in two Moroccan strains of *Verticillium albo-atrum* (microsclerotial forms).

10.15 - F. M. McGEARY and A. C. HASTIE (U.K.): Hybridisation of *Verticillium albo-atrum* strains from tomato and lucerne.

10.30 - H. LAHLOU and C. BOISSON (Maroc): Pathogenicity variations in one tomato isolate of *Verticillium dahliae*.

10.45 - J. E. PUHALLA and M. HUMMEL (U.S.A.): Further studies on heterokaryon-compatible populations within *Verticillium dahliae*.

11.00 - Coffea & Tea.

Session IV: Pathogen-disease physiology
Chairman : H. MUSSELL

11.30 - C. H. BECKMAN (U.S.A.): The succession of interactions between host plants and vascular parasites.

12.00 - J. M. FERRARI and N. J. WHITNEY (Canada): Wound periderm formation of potato tubers in relation to resistance to Verticillium wilt.

12.15 - A. NACHMIAS and J. KRIKUN (Israel): The role of toxins in the pathogenesis of *Verticillium dahliae* on potato.

13.00 - Lunch.

Afternoon:

14.30 - A. PUGIN (France): Effects on membrane permeability of two glycopeptides produced by *Phialophora cinerescens* (Wr.) Van Beyma.

14.15 - M. MOREAU and A. M. CATESSON (France): Behaviour of the cells of the xylem parenchyme after a vascular stress.

15.15 - J. ROBB, L. BUSCH and A. SMITH (Canada): The range of foliar symptom expression in Verticillium-infected hosts: an ultrastructure survey.

15.30 - T. BOISOT, J. DUBOUCHET and M. PERESSE (France): Relationship between activity changes of multiple molecular forms of the peroxidase and development of *Dianthus caryophyllus* after infection with *Phialophora cinerescens.*

15.45 - Coffea & Tea.

16.15 - *Session V:* Posters.

J. M. CLARKSON and J. B. HEALE (U.K.): Genetical studies of strains of *Verticillium albo-atrum* attacking hop.

G. P. HARRIS and J. B. HEALE (U.K.): Studies in differential resistance in lucerne cultivars inoculated with strains of *Verticillium albo-atrum.*

M. MOREAU and A. M. CATESSON (France): Behaviour of the cells of the xylem parenchyme after a vascular stress.

N. J. WHITNEY and K. S. HUNG (Canada): Studies on germination of spores of *Verticillium albo-atrum.*

Thursday, August 27

Morning:

8.00 - Departure for visit to prehistoric cave-dwellings of the town of Matera (60 km from Bari) and to the agricultural area and experimental fields near Policoro (150 km from Bari).

Evening:

21.00 - Return to Bari.

Friday, August 28

Morning:

- *Session VI:* Pathogen-disease physiology (continued)
- *Chairman :* H. MUSSELL

9.00 M. E. MACE and A. A. BELL (U.S.A.): Biotic and abiotic elicitation of terpenoid aldehyde phythoalexins in cotton.

9.15 - P. F. S. STREET and R. M. COOPER (U.K.): Possible causes and sites of vascular occlusion in tomato infected with *Verticillium albo-atrum.*

9.30 - J. M. MILTON and O. M. O. ONUORAH (U.K.): The elicitation of lucerne (alfalfa-*Medicago sativa* L.) phytoalexins by *Verticillium* spp.

9.45 - H. MUSSELL and P. STILWELL (U.S.A.): Possible origin and participation of toxin in Verticillium wilt of tomato.

10.00 - Coffea & Tea.

Session VII: Sources and genetics of resistance
Chairman : J. GONDRAN

10.30 - S. WILHELM (U.S.A.): Sources and genetics of Verticillium wilt resistance in major crops.

11.00 - Y. PINKAS and A. KARIV (Israel): Screening for resistance against *Verticillium dahliae* in avocado rootstocks.

11.15 - D. P. GUPTA (India): Histological changes during the Verticillium wilting syndrome in susceptible and tolerant lucerne cultivars.

11.30 - C. D. BISHOP and R. M. COOPER (U.K.): An ultrastructural study of resistance of tomato to *Verticillium albo-atrum.*

11.45 - J. GONDRAND (France): Stability of the Verticillium wilt resistance of lucerne cultivars.

12.00 - J. R. DAVIS, J. J. PAVEK and D. L. CORSINI (U.S.A.): Stability of field resistance to *Verticillium dahliae* in potato cultivars and relationship to soilborne inoculum.

12.15 - L. V. BUSCH, E. A. SMITH and J. ROBB (Canada): Verticillium wilt of lucerne. Some results with a North American isolate.

12.30 - M. CIRULLI and F. CICCARESE (Italy): Progress in the search for Verticillium wilt resistant eggplant.

13.00 - Lunch.

Afternoon:

Session VIII: Chemical control
Chairman : E. C. TJAMOS

15.00 - P. W. TALBOYS (U.K.): Chemical control of Verticillium wilts.

15.30 - S. KOCATURK (Turkey): Studies on the chemical and biological control of Verticillium wilt disease.

15.45 - E. C. TJAMOS, A. CHITZANIDIS and E. KORNAROS (Greece): Failure of two growth retardants to suppress Verticillium wilt symptoms and increase yield in cotton field trials in Greece.

16.00 - J. KRIKUN, D. ORION and A. NACHMIAS (Israel): Integrated control of *V. dahliae* in potato.

16.15 - Coffea & Tea.

16.45 - C. CHRISTIAS, E. TJAMOS, C. ZIOUDROU and E. KORNAROS (Greece): *In vitro* inhibition of microsclerotia formation by cysteamine hydrochloride in *Verticillium dahliae.*

17.00 - Y. BEN-YEPHET, E. SITI and Z. FRANK (Israel): Penetration of metham-sodium into loess soil as measured by its effect on the viability of *Verticillium dahliae* microsclerotia.

17.15 - *Closing session*
Chairman: M. CIRULLI

17.45 - Members disperse or depart on post-Symposium organized professional tours.

SUMMARIES

The Succession of Interactions Between Host Plants and Vascular Parasites

C. H. BECKMAN

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Any discussion of host-parasite relationships must consider each interactant in a dynamic state of action and reaction. The work of Cooper and Wood, showing the sequence of enzyme inductions that occurs when *Verticillium* is grown on host cell wall preparations, is an outstanding example of this dynamic nature of the interactions. Analysing the many interactions that can take place between two variable interactants in a variable environment must inevitably, however, become extremely complex.

We may reduce this problem of complexity by sub-dividing these interactions into recognition, response, and termination phases. This is most easily observed, within the host, in the resistant reaction in which the interactions appear to be terminated within 3-5 days, and in which the outcome may be determined within a much shorter time. In the susceptible interactions, the situation becomes far more complex because the pathogen has the capacity to circumvent or inhibit recognition, response, or termination. Then the interaction becomes protracted over a far larger time-space frame. It seems best, therefore, to study these interactions with a systems approach, beginning with the initial contact and following the succession of events, step-by-step, in short time-space sequences.

These events are reviewed and compared, as they progress in resistant vs. susceptible interactions, in an attempt to provide a framework for subsequent presentations and discussion. The sequence begins in the soil with the recognition, by the pathogen, of a nutrient base in plant rhizospheres. It continues with the initial physical contact between potential host and potential pathogen wherein recognition of each by the other occurs; continues with enzyme and toxin production by the parasite and greatly enhanced synthesis within host tissues of hormones, cellular constituents and phytoalexins. The course of these interactions is discussed in terms of pathogenicity of the parasite and resistance of the host.

Penetration of Metham-Sodium into Loess Soil as Measured by its Effect on the Viability of Verticillium dahliae Microsclerotia

Y. BEN-YEPHET*, E. SITI and Z. FRANK***

** Division of Plant Pathology, Agricultural Research Organization, the Volcani Centre, Bet Dagan and*
*** Extension Service, Israel Ministry of Agriculture, Be'er Sheva, Israel*

The method of metham application at low, constant concentrations through a sprinkler irrigation system was developed by J. Krikun and co-workers. Since the released toxicant methylisothiocyanate (MIT) is known to be retarded by soil colloids, it was assumed that a split application - concentrate first, followed by dilute solution - would translocate sufficient MIT to the deeper layers.

Equal doses of metham were applied to soil colums in three ways: (i) drenching with a concentrate (the whole dose applied onto the column, followed by watering to field capacity); (ii) irrigating with a dilute solution of the fungicide to field capacity; and (iii) combined applications of the split dose (50% of metham concentrate first, drenched by a dilute solution of the rest). *Verticillium dahliae* microsclerotia (MS) were introduced into the soil profile to assess the presence of MIT at different dephts. Penetration of MIT was best with the combined application.

Microplots containing inserted MS were irrigated to field capacity at 40 cm depth using the dilute *versus* combined applications, and at four metham (32% a.i.) doses (600, 800, 1000 and 1200 l ha^{-1}). In the combined application half the dose was applied in 12.5% of the irrigation water, followed by the other half diluted in 87.5% of the water. Increasing the metham dose led to a deeper penetration of the toxicant in the combined application than in the dilute one.

In two *Verticillium dahliae*-infested fields, additional MS were inserted to different depths. Metham, at 1000 l ha^{-1}, was applied by sprinkler irrigation to wet the top 40-cm soil layer to field capacity. In the combined treatments, 65% or 70% of the toxicant was injected into 15% of the irrigation water and the rest into the following 85%. Seventeen days after treatment, cv 'Spunta' potatoes were planted and inserted MS recovered. Comparison with the control showed that the dilute, unsplit application killed all MS to depth of 10-20 cm only, whereas the combined applications, killed all MS to a depth of 30-40 cm. In the combined applications plant infection was remarkably reduced.

An Ultrastructural Study of Resistance of Tomato to Verticillium albo-atrum

C. D. BISHOP * and R. M. COOPER **

** Department of Biology, Liverpool Polytechnic, Liverpool, and ** School of Biological Sciences, University of Bath, Bath, U. K.*

Transmission- and scanning-electron microscopy were used to study the mechanisms of resistance of tomato to *Verticillium albo-atrum.*

Epidermal and cortical root cells of resistant and susceptible varieties reacted similarly to invasion; intercellular penetration elicited host wall appositions adjacent to penetrating hyphae and subsequent wall penetration resulted in penetration papillae (lignitubers). The latter were apparently effective in preventing further hyphal growth, although the frequency of their formation was similar in both resistant and susceptible tomato roots.

A differential expression of varietal resistance occurred at the endodermis. Penetration of susceptible endodermal cells resulted in cell death with no apparent restriction of invading hyphae, but similar damage to resistant cells induced the accumulation of an electron-opaque material. Hyphae remained within such cells, swelled to 2-3 fold their normal diameter and were highly vacuolate with granular disrupted cytoplasm. However, some hyphae entered the stele either by intercellular growth between endodermal cells or more commonly, by longitudinal growth from the root tip, which has no differentiated endodermis. Thus, the putative line of resistance of the endodermis in the resistant variety could be bypassed, although it appeared effective in limiting colonisation of the stele of mature roots.

Systemic distribution of the pathogen was rapid in the xylem of susceptible varieties but was limited by rapid tylosis in the resistant cultivar. Tyloses were formed, following the formation of 'protective layers' within xylem parenchyma cells and dissolution of pit membranes, by expansion of xylem parenchyma cells into vessel lumina. Cell expansion was accompanied by an increase in vacuolar volume and a simultaneous accumulation of electron-opaque material within the enlarged vacuoles. In the resistant variety accumulation of electron-opaque material was also common in xylem parenchyma cells not involved in tylosis. This may indicate an increase in secondary metabolism within such cells, possibly related to phytoalexin synthesis.

These reactions will be briefly compared with those in some other vascular wilt diseases.

Relationship Between Activity Changes of Multiple Molecular Forms of the Peroxidase and Development of Dianthus caryophyllus After Infection with Phialophora cinerescens

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*** Plant Biology, State University, F-29283 Brest, France*

Six-week-old carnations infected by watering their culture medium with a suspension of spores of *Phialophora cinerescens,* subsequently exhibited, among other metabolic alterations, an enhanced lignification in the basal part of the stem and a reduced growth of its apical part. These modifications were linked to the development of *P. cinerescens* in the stem. They were related to changes in activity of the different molecular forms of cell wall and soluble peroxidases. For an 11-week period following the infection, 3 main phases were distinguished.

During the 1st one (1 to 2 weeks), activities of all the peroxidases were increased in the lower part of the stem where spores of *P. cinerescens* were located (local effect) and in its middle part, i.e. in advance of the pathogen (distal effect).

The 2nd phase was characterized by the decrease of the previously enhanced peroxidase activities, which thus became similar to those of the controls. At this time, infected carnations still looked like controls.

The 3rd phase began 4 to 5 weeks after infection and coincided with the vertical progression of the mycelium which first arrived in the middle stem. Most of the peroxidase activities attained a level which denoted an accelerated senescence, especially in the middle stem (local effect) and in its upper part (effect at a distance). In parallel with these facts, it was found that lignification was enhanced in the basal part of the stem whereas growth was strongly decreased in its apical part. As far as different stem tissues were concerned, it appeared that activity of the soluble peroxidases increased in every tissue (horizontal effect) and more especially in xylem and pith. In both these tissues, activity of the bulk of the covalently bound cell wall peroxidases was also shown strongly to increase. These modulations may be similarly related to compounds produced *in vitro* and *in vivo* by *P. cinerescens,* and more especially to two glycopeptides of low molecular weight.

Thus, after infection with P. *cinerescens,* the peroxidase activity changes which occurred in the stem part still able to continue growing (like the upper part) appear probably to be involved in the growth reduction, whereas those which occurred in the stem part, the growth of which was complete (like the lower part), seem to be involved in lignification enhancement.

Intraclonal Morphological Variations in Two Moroccan Strains of Verticillium albo-atrum (Microsclerotial Forms)

C. BOISSON and H. LAHLOU

Laboratoire de Mycologie - Faculté des Sciences - BP 1014, Rabat, Morocco

Morphological variability was studied with two monosporous clones of *Verticillium albo-atrum* obtained from a tomato isolate (n. 1) and from an avocado isolate (n. 3). The microconidial descendance obtained from young cultures was homogeneous: all thalli showed wild phenotypes. On the other hand, the conidial descendance obtained from old cultures was heterogeneous: besides the wild phenotype, variant phenotypes appeared in sometimes high percentages (up to 93%).

These variants were of two types:

— in the first type the morphology of microsclerotia was modified: big microsclerotia variants from isolate 1, « halo » variants from isolate 3, with small elongated microsclerotia which showed almost no budding;

— in the second type (hyaline variants), the sclerogenesis was very late and weak, sometimes absent; conidiogenesis was occasionally altered in the same way.

These variants remained stable after mycelial or microconidial regeneration from young cultures. However, the variants of the first type could be altered again, by microconidial regeneration from older cultures. In this case, a variable percentage of hyalin variants appeared, and, in these variants, there was never reversion to the wild phenotype. Hyaline thalli were always stable and never reverted to the wild type.

It therefore seems that morphological variations always arise in the same way: gradual loss of the ability to form microsclerotia and eventually even microconidia.

Verticillium Wilt of Lucerne - Some Results with a North American Isolate

L. V. BUSCH *, E. A. SMITH ** and J. ROBB *

** Department of Botany and Genetics, and ** Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada*

Disease ratings of 4 European lucerne cultivars (Maris Kabul, Vertus, Maris Phoenix and Du Puits), reported by Lusignan, France and Cambridge, G. B., were similar to those obtained at Guelph, Canada, but higher than those reported by Aberystwyth, G. B. The culture used at Guelph was the most virulent of several isolates supplied by J. W. Sheppard, Ottawa. It was isolated from infested alfalfa seed grown in Washington State, U.S.A. These results would suggest that the North American isolates are similar in virulence to those found in Europe and disagree with a recent report from the U. K. suggesting the converse.

Resistance present in 36 annual *Medicago* species and 27 perennial species tested was not related to the area of origin of the individual species. There was more resistance present in the annual *Medicago* species than in the perennial ones tested. Many of the species were very susceptible with most of the plants completely dead within 10 days of inoculation.

Other clovers and several weeds proved to be susceptible to the isolate used and it is considered that these hosts could act as reservoirs for the lucerne strain of *Verticillium albo-atrum*.

In Vitro **Inhibition of Microsclerotia Formation by Cysteamine Hydrochioride in** Verticillium dahliae

C. CHRISTIAS *, E. TJAMOS **, C. ZIOUDROU * and E. KORNAROS **

** Nuclear Research Center « Demokritos », Aghia Paraskevi, and*
*** Benaki Phytopathological Institute, Kifissia, Athens, Greece*

Certain sulfhydryl compounds have been known to inhibit the formation of sclerotia. In *Sclerotium rolfsii* sclerotium formation was completely inhibited by 2-mercaptoethanol as well as by other mercaptans, whereas mycelial growth was not affected. Any chemical compound which might inhibit the formation of sclerotia is of considerable interest since it may have potential value for disease control. In this communication we report a specific inhibitory action of the compound cysteamine hydrochloride on the formation of microsclerotia of *Verticillium dahliae.*

A cotton isolate was grown on standard PDA plates to which the inhibitor had been added at concentrations of 3, 6 and 12mM. The plates were inoculated with 0.2 ml of a conidial suspension evenly distributed on the agar surface and then incubated at 22°C. Observations were made 4, 20 and 30 days after inoculation.

In control plates, globular and linear microsclerotia appeared 4 days after inoculation. A massive production of pigmented microsclerotia occurred 20 days after inoculation and plates were completely black ten days later. In cultures grown at 3mM no microsclerotia were formed after 4 days and only rarely after 20 days inoculation. Numerous pigmented microsclerotia were observed 30 days after inoculation situated mostly at the periphery of the plates. There was no retardation of mycelial growth. Mycelium grown at 6mM produced neither microsclerotia nor primordia. There was some retardation of growth as judged by reduced aerial mycelial growth. A prominent feature was the formation of thin spiral hyphae. Typical verticillate conidiophores were present. Growth at 12mM was the same as in 6mM except that no verticillate conidiophores were present.

The specific inhibitory action of cysteamine-HCl on microsclerotium formation, apart from its value in the study of the process of microsclerotium formation, may have implications for the control of this pathogen.

Progress in the Search for Verticillium Wilt Resistant Eggplant

M. CIRULLI and F. CICCARESE

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Verticillium wilt (*Verticillium dahliae* Kleb.) is a highly destructive disease wherever eggplant *(Solanum melongena L.)* is grown. Cultural practices, grafting on *Ve*-resistant tomato rootstocks and soil disinfestation are ineffective or limited by cost and/or skill. The most effective and economical control can be obtained by the use of resistant varieties. Although diverse expressions of resistance have been reported to occur in eggplant, no progress has been made in developing commercial varieties resistant to *Verticillium*. The present paper describes the screening of eggplant accessions for resistance to *V. dahliae,* the development and evaluation of S2 selfed resistant progenies, the nature of the inheritance of resistance, and the achievements made in breeding for commercial resistant varieties.

A total of 116 eggplant accessions were tested for resistance to *V. dahliae* in the field using two methods of disease assessment that were based on external and internal disease symptoms. Ratings of external symptoms were made using a scale of 0 to 5 in which 0 = absence of symptoms (i.e. leaf mottling, flaccidity and necroses, and plant stunting) and 5 = dead plant. Internal symptoms were evaluated on a transverse section of the main stem using a scale of 0 to 5 in which 0 = absence of vascular discoloration and 5 = dead plant. Each plant was scored for both external symptoms and vascular discoloration, and the amount of disease (rate of diseased plants and severity index) was calculated for each accessions.

No accessions possessed high resistance. Some accessions showed good levels of resistance which was characterized by a lower rate of diseased plants and in particular, by disease severity values, for both external symptoms and vascular discoloration, that were significantly lower than those for the susceptible Florida Market.

Resistance ratings based on external symptoms, did not always coincide with those for vascular discoloration on resistant accessions, whereas the two methods of disease appraisal coincided in highly susceptible entries. Resistance found in the same original eggplant accessions (So) was transmitted in selfed S_1 and S_2 progenies which, in general, did not differ from their parental accessions in terms of the degree of resistance.

Inheritance studies made on a 5 x 5 diallel mating design, involving four resistant S_2 selections (denominated R1, R2, R3 and R4) and the susceptible Florida Market, showed that the genetic control of resistance is partially dominant. Progenies from S_2 resistant selections crossed to susceptible commercial varieties proved to posses good levels of resistance to Verticillium wilt and valuable horticultural characters.

Estimation of the Distribution and Pathogenicity of Verticillium Population in Soils Using Selective Baitplants

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Quantitative assessment of *Verticillium* in field soils by use of selective isolation techniques gave no direct indications of the possible damage in consecutive crops. Experiments on biological detection of *Verticillium* were carried out.

The use of a selective indicator plant (baitplant) - sown or planted in a soil sample - may give more relevant information about pathogenicity. Two problems were studied:

1. Which is the most useful and reliable baitplant to determine the inoculum density and pathogenicity of *Verticillium* populations in relation to plants which cannot be used themselves as test plants?
   Soil substrates were artificially infested with different inoculum densities of *Verticillium dahliae* and *V. albo-atrum*. Baitplants flax, eggplant and *Impatiens balsamina* L. were sown in this soil substrate. Six weeks after sowing, the seedlings were analysed for the presence of *Verticillium*. For each testplant and *Verticillium* species, a correlation was drawn between the number of infested baitplants and the inoculum density. From these experiments, flax appeared to be the most promising bait.

2. Is it possible, by using soil amendments, to reduce the incubation time after sowing the baitplant from 6 to 3 weeks?
   The original soil was enriched by: 1. Czapek Dox (33,4 g/l + biotine (15µg/l) and perlite (1:6 volume by volume); 2. oat meal; and 3. Draff. Flax seedlings were analysed 2, 3 and 4 weeks after sowing. The number of the infested plants 2 and 3 weeks after sowing, however, was lower in the case of soil amendment than for the unenriched soil.

Stability of Field Resistance to Verticillium dahliae in Potato Cultivars and Relationship to Soilborne Inoculum

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Potato cultivars with a history of Verticillium field-resistance were compared with commonly-grown, susceptible cultivars by continuously cropping for four consecutive years. Results indicate stability for field resistance to *Verticillium dahliae* Kleb. by two cultivars. Lines A66107-51 (-51) and A68113-4 (-4) have shown consistently significantly ($P = 0.05$) less wilt and less colonization by *V. dahliae* in apical stem tissue than susceptible cultivars [Russet Burbank (RB) and Butte (B)]. Each year the wilt severity for either -51 or -4 has remained negligible ($<1.0\%$). In contrast, relative wilt severities for either RB or B have consistently exceeded 39.0%. These data are further corroborated by symptoms of vascular discoloration and *V. dahliae* colonization at base of stems. Throughout these four years of cropping, foliar wilt symptoms, vascular discoloration, and *V. dahliae* colonization for Targhee (T) have generally been between the susceptible and resistant extremes. With continuous cropping, cultivars with highest field resistance (-51 and -4) have maintained the highest yields along with acceptable quality. Yields with -51 have ranged from 28 to 110% higher than RB while yield increases with -4 have ranged from 38 to 142% higher (.05 P significance). The percentage of root infection by *V. dahliae* was observed to be significantly ($P = 0.05$) less with -51 than with either RB or B. Although the same trend for less root infection existed for T and -4, differences were not significant.

Results further suggest decreases in *V. dahliae* soil populations that are related to cultivar resistance. After two and three respective years of continuous cropping, *V. dahliae* populations in soil (sampled annually prior to planting) correlated ($P = 0.01$) with wilt symptoms among all cultivars ($R^2 = 0.37$ for each consecutive year). After two years of cropping. *V. dahliae* soil populations were lower ($P = 0.05$) in plots where either T, -51, or -4 were grown than in plots where RB was grown.

Epidemiology and Ecology of Diseases Caused by Verticillium Species - With Emphasis on Verticillium Wilt of Cotton

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Diseases caused by *Verticillium* species range from those that affect other fungi to those of at least 45 plant families. In many of these diseases, infection of the vascular tissue leads to pathogenesis and various expression of wilt symptoms. However, plant roots often are infected by *Verticillium* species but without development of macroscopic disease symptoms.

Numerous epidemiological and ecological conditions influence the distribution, establishment and pathogencity of *Verticillium* species. Depending on the plant host, the spread of *Verticillium* propagules may occur on seed, in other plant parts, or as movement in soil as free propagules, or in plant debris. Characteristically, *Verticillium* species, especially *V. dahliae* and *V. albo-atrum,* are nonaggressive soil residents which seldom venture more than a few millimeters from their propagule base; their longivety in the soil is largely dependent on interactions with plant roots or other plant parts. Host specialization, depending on the species of *Verticillium* and populations within species, may be broadly or narrowly defined.

It appears, however, that colonization of host tissue does not always result in disease. Pathogenicity of *Verticillium* species is conditioned not only by host-pathogen interactions but is also responsive to ecological factors, especially temperature. Epidemiology of Verticillium wilt diseases involves the combined effects of plant water relations, soil nutrients, especially nitrogen and potassium salts, soil and air temperatures, and plant and root densities. Disease development, however, is most heavily influenced by concentrations of *Verticillium* propagules in the soil, a condition made worse by the continuous culture of Verticillium wilt-susceptible crops. For example, in soils continuously planted to cotton *(Gossypium hirsutum),* the concentration of propagules of *V. dahliae* in two field sites increased on the average of 13 to 15 propagules/gram of soil/year. In these field sites where mixtures of defoliating and nondefoliating pathotypes were present, the concentrations of propagules leveled off in the range of 60 to 70 propagules/gram of soil. In fields containing high proportions of the defoliating pathotype of *V. dahliae,* concentrations approaching 300 propagules/gram of soil have been found. These numbers may seem unusually high for propagule concentrations of *V. dahliae* in tomato fields but low for potato fields with severe Verticillium wilt problems.

In cotton fields, disease progress curves showed a highly significant relationship between foliar symptom development and soil-borne propagule densities of *V. dahliae.* Moreover, increasing slopes of disease progress curves were proportional to propagule densities at less than 40 propagules/gram of soil. Infection of cotton roots by *V. dahliae* is usually indicated by vascular browning;

however, the incidence of foliar symptoms is always less than the percentage of cotton plants with vascular browning. Lint fiber yields are directly related to the extent of plant growth and development before the onset of foliar symptoms. Plant growth analyses have shown that one of the first major symptoms in Verticillium-infected plants is a reduction in internode elongation that is apparent about two weeks before the onset of foliar disease symptoms. Shedding of flower buds also is associated with onset of foliar symptoms, but bolls usually remain attached. Verticillium wilt of cotton as well as other diseases caused by *Verticillium* species have been managed mainly through crop rotations, resistant or tolerant cultivars of crop plants and, more recently, by soil solarization. In the latter method, soil heating causes the death of *Verticillium* propagules which are highly sensitive to high temperature/time effects. In addition, an evaluation of these various plant disease control methods has pointed to changes in microbial populations in soil as a possible basis for the disease control achieved.

Extracellular Pectolytic Enzymes of Verticillium albo-atrum and Verticillium dahliae

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Verticillium albo-atrum, causing stalk-rot of banana fruits, and *V. dahliae,* causing wilt of cotton in India, were examined for their extra-cellular pectolytic enzyme production under varying cultural conditions. *V. albo-atrum* produced PAL (pectic acid lyase), PL (pectin lyase) and PMG (pectin methyl galacturonase) by 7th day on Richard's solution containing NaPP (sodium polypectate) as exclusive carbon source. With pectin, however, only PL was detectable in TBA (thiobarbituric acid) test. Same were the observations with Czapek Dox and potato-pectic media. The production of PMG was repressed by various sugars except galactose. Lactose repressed PAL in addition to PMG, but it induced the synthesis of an additional enzyme - PG (polygalacturonase), not produced in presence of NaPP alone.

V. dahliae too elaborated both hydrolytic and trans-eliminative enzymes and showed a distinct temporal sequence of synthesis. On Richard's -NaPP medium, the enzymes were different on 7th and 14th day of the incubation period.

The constitutive production of pectolytic enzymes was noted for both the organisms: *V. albo-atrum* on Richard's solution containing sucrose, and *V. dahliae* on Czapek Dox solution containing glucose. These enzymes were lyases. The important aspect of regulation of pectolytic enzymes synthesis under the influence of different sugars, amino acids, phenols, growth hormones etc. will be presented on the basis of our findings.

Administrative Measures for Control of Progressive Verticillium Wilt of Hops in England

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Wilt of hops *(Humulus lupulus L.)* caused by *Verticillium albo-atrum* Reinke et Berth. was recorded in the south-east of England (Kent) in 1924 and in the West Midlands (Herefordshire) in 1930. Two forms of the disease were distinguished: one which seldom killed the plant and caused symptoms varying in intensity from year to year (« fluctuating »), and a more virulent (« progressive ») form which killed plants and spread progressively through the plantations attacked.

Spread to new areas was frequently by planting infected or contaminated hop sets taken from infected hop plantations. The Ministry of Agriculture, Fisheries and Food (the Ministry) therefore introduced in 1943 a voluntary scheme for the inspection and certification of hop plantations for freedom from symptoms of Verticillium wilt. New methods of propagation, firstly by layering bines in special nurseries, and later by softwood cuttings and rooting under mist, emphasized the importance of ensuring that mother plants were healthy. By the time the A-plus certification scheme was introduced in 1955, pathogen-tested clones were being propagated by specialist growers far removed from the wilt infested areas of commercial hop production.

Continued spread of the disease in south-eastern England prompted legislative measures. The Progressive Wilt Disease of Hops Order 1947 prohibited the sale of hop plants from land known to be affected by progressive Verticillium wilt and obliged growers who suspected the occurrence of the disease on their land to notify the Ministry. The revisions of the Order in 1953, 1957, 1965 and 1978 provided for more comprehensive compulsory control measures and governed the movement of planting material.

It is believed that certification and legislative measures have slowed considerably the spread of progressive Verticillium wilt of hops in England. However, it is now accepted that the disease is not eradicable from a specified area, comprising East Sussex and parts of Kent and West Sussex. Efforts are now concentrated on containing the disease in eastern Kent and on eradicating it wherever possible elsewhere.

Occurrence and Distribution of Verticillium Wilt of Sunflower in Egypt

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The cultivated sunflower (*Helianthus annuus* L.) is one of the most important annual crops in the world grown for edible oil. Egypt's rapidly increasing population and great demand for oil-seeds and vegetable oils led to the need to increase both production and import. As sunflower cultivation has expanded, diseases have increased in number and severity. Surveys on sunflower were carried out in Egypt in August 1977, a time when most sunflower diseases have reached a climax. An epiphytotic disease of leaf mottle disease (= Verticillium wilt) on sunflower was first discovered in a small plantation of the grey-stripped sunflower variety following a potato crop. *Verticillium dahliae* was identified as the causal pathogen. The initial infection was estimated at about 15% of the plants; however, late in the season disease losses were estimated to reach 90%. During the 1978 season, the same condition was observed and caused moderate to severe yield losses in the principal sunflower producing areas of Giza Governatorate south of Egypt, particularly in the grey-stripped variety in lighter soil areas and in fields following potatoes or beans. Symptoms are often manifest, first on the lower leaves and then develop successively on the leaves higher on stem. The leaves show prominent yellows, interveinal patches usually first in the centre or near the periphery of the leaf. Black, streaky patches occur at the base on the stem. Cross sections of the lower stem show a brown discoloration of the vascular system. From pathogenicity studies in the greenhouse it was concluded that the grey-stripped cultivar was highly susceptible, while Giza 1, a white local variety, showed moderate infection.

Assessment of Methods for Measuring Losses Caused by *Verticillium dahliae* in Cotton

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Although estimates of losses caused by *Verticillium dahliae* in cotton abound, there are no unequivocal data available relating inoculum level (microsclerotia/g) in soil to disease incidence, yield and fibre quality. In those studies which have been published, measurements of yield loss are confounded by other influences such as cultivar, and rotational or fumigation treatments. Research into experimental techniques and design for measuring yield loss will be described. Methods for measuring the effect of *V. dahliae* on various components of yield (boll number, boll weight, seedcotton/boll, and lint/boll) will also be described.

Wound Periderm Formation of Potato Tubers in Relation to Resistance to Verticillium wilt

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The formation of wound periderm as a factor contributing to Verticillium wilt resistance of potato was examined. Wound periderm formation was studied in three cultivars of potato plants *(Solanum tuberosum):* Kennebec, Houma, and F61019 which have low, high and high resistance to *Verticillium albo-atrum* Reinke et Berth., respectively. Inoculation of the seed pieces was undertaken immediately after cutting, and at 2, 5, 8, and 11 days after cutting. Histochemical techniques were employed to compare phenomena of host-parasite interaction at the wound periderm.

Quantitative Measurement of Losses Caused by Verticillium dahliae in Cotton

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During the summers of 1978-79 and 1979-80, experiments were established in the Namoi Valley, New South Wales, to measure losses caused by *Verticillium dahliae* in cotton, and to relate inoculum density in soil to disease incidence, and yield. The trials were randomised complete blocks of 5 replications and 6 inoculum levels. These were located in a field which had a low level (less than 4 microsclerotia/g) of natural inoculum.

During the winter months each plot was infested with microsclerotia (ms) produced on a mixture of milled oat grain and wheat chaff. Soil samples were collected in late spring and assayed for *V. dahliae* (ms/g). The plots, comprising 6 rows of cotton, each 3 m long, were thinned to a uniform plant density, and each plant was identified by a numbered tag. The plants were assessed for foliar symptoms of wilt at intervals from first flower to late boll set. Stems were cut after harvest and examined for internal discolouration that is characteristic of infection by *V. dahliae*. Yield data were obtained by hand picking.

Although neither season favoured the rapid development of Verticillium wilt, the results were complementary. The relationships between inoculum levels, % infected plants, average times of infection, and yield loss were examined. Incorporation of inoculum in plots provided a range of inoculum levels from 1 ms/g soil in the uninoculated controls to 145 ms/g soil in the most heavily infested plots in 1978-79, and from 2 to 32 ms/g soil in 1979-80. Analysis of the relationship between disease incidence and yield showed that % diseased plants after harvest, as determined by vascular examination, was the best predictor of yield. As the level of inoculum increased, disease incidence increased significantly from 6% in the controls to 80% in the highest treatments in 1978-79, and from 7% to 84% in 1979-80, and the average times of infection were earlier. There was a significant negative correlation between disease incidence and yield in both seasons. Based on production costs and crop values in commercial situations, the highest losses recorded translated to profit losses of about A$180/ha and A$210/ha in 1978-79 and 1979-80 respectively.

Stability of the Verticillium Wilt Resistance of Lucerne Cultivars

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Three trials were carried out to ascertain whether the geographic origin of isolates of *Verticillium* affects the susceptibility of lucerne cultivars.

In the first trial the correlation coefficients between the scores of natural infection of 20 cultivars in Rennes (France) and the result of the same cultivars artificially infected with different methods in Lusignan (France), Aberystwyth (G. B.) and Cambridge (G. B.) were significant.

In the second trial, 4 lucerne cultivars (Vertus, Verneuil, Luciole, Du Puits), were artificially infected by pruning the stems with infected scissors. The isolates of *Verticillium albo-atrum* came from 4 regions of France (Poitiers, Champagne, Normandy, Burgundy). The interaction between the fungus isolates and the lucerne cultivars was not significant.

In the third trial, 5 lucerne cultivars (Maris Kabul, Vertus, Verneuil, Europe, Du Puits were similary infected with 5 isolates of *V. albo-atrum* from France, Spain and Switzerland. The interaction between the fungus isolates and the lucerne cultivars was significant but the variance interaction was low in comparison with the variance of the main effect (cultivars). The only change in the ranking of cultivars was brought by the Swiss isolate which lowered the resistance of Maris Kabul to the level of Vertus.

It is concluded that the variation in pathogenicity of the isolates does not affect markedly the stability of the resistance of lucerne cultivars.

Variations in Strains and Species of *Verticillium*

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The production of polygalacturonase (PG), pectin-methyl-esterase (PME) and carboxymethylcellulase (C_x) by six isolates of *Verticillium albo-atrum* Reinke et Berth., six isolates of *V. dahliae* Kleb. and single isolates each of *V. tricorpus, V. theobromae, V. nigrescens, V. nubilum,* and *V. lateritium* is variable when glucose, pectin and carboxymethylcellulose are used separately as carbon sources in culture under constant conditions of medium and temperature in the dark. Some strains and species do not show PG-activity although thiobarbituric acid-detectable substances are present in their enzyme reaction mixture. No C_x- activity was detectable in any culture in the presence of either glucose or pectin. All strains and species produce C_x only in the presence of cellulosic substances. Thus in all these strains and species C_x is inducible and PG and PME are produced constitutively.

All strains and species grown under constant conditions of medium and temperature in either dark or light vary in the production of spore, protein, pigment and dry weight of mycelium. Partial ultraviolet radiation suppresses the production of spores and pigments differently in all cases. These differences provide a basis for the designation of strains and species.

Histological Changes During the Verticillium Wilting Sindrome in Susceptible and Tolerant Lucerne Cultivars

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Verticillium-susceptible (Du Puits) and tolerant (Swedish cv) cultivars are characterized by a number of host reactions. The Swedish cultivar showed resistance to disease expression at all levels of inoculum concentration with a lucerne isolate of *Verticillium albo-atrum* Reinke et Berth. Delayed root penetration and slight colonization of the shoot are correlated with heavy lignification and suberization of the root-endodermis and frequent formation of tylosis in the vessels of the shoot. Tylosis formation was not in accordance with disease expression. None of the infected plants wilted seriously or died and new shoot-growth started immediately after infection to produce a large number of secondary vascular tissues and bast fibres frequently containing fungal hyphae surrounded by dividing cells.

The cultivar Du Puits was severely diseased at the lowest level of inoculum. Vascular browning was always observed. Primary and secondary xylem of all infected plants contained hyphae and brown staining gels which blocked a large number of vessels. In some cases branched hyphae and possibly conidiophore formation were observed. In Du Puits susceptibility is directly proportional to the amount of hyphae present in the vessels, while in Swedish cultivar a physiological resistance mechanism independent of the degree of colonization appeared to operate.

The Genetics of Verticillium

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Genetic recombination through heterokaryosis and the parasexual cycle is well known from laboratory studies with pathogenic *Verticillium* species. Significant advances deriving from the application of these and other techniques to the investigation of both natural variation and basic cellular processes have been reported in the past 5 years. Puhalla (1979), using induced colour mutants, showed that wild populations of *V. dahliae* contain heterokaryon incompatible strains, and that such evidence might reveal information about the ancestry of new pathogenic strains. Evidence from several laboratory studies involving diauxotrophic mutants derived from different host strains of *V. albo-atrum* (Hastie, 1964, 1973, Typas and Heale, 1976, McGeary, 1980, Clarkson and Heale unpublished) suggests that the slight degree of incompatibility sometimes encountered during « forced heterokaryon » tests was unlikely to be a major factor in preventing gene-flow, at least in this species. The genetic control of the natural dark/hyaline dimorphism of *V. albo-atrum* has been further investigated by Typas and Heale (1979) who showed, by micro-injection of various cell fractions, that mitochondrial preparations contain at least one factor controlling dark pigmentation. Basic cell processes including the nuclear cycle, conidium germination and hyphal growth are also being investigated. Typas and Heale (1980) used micro-densitometry to investigate both the nuclear cycle time and also the « ploidy » of somatic cells. Hastie (unpublished) has searched for temperature-sensitive mutants of *V. albo-atrum* affecting conidium germination and hyphal growth with a view to analysing genetic control of the respective processes. No mutations specifically affecting conidium germination have been detected. That result will be discussed in relation to the discovery of conidium-specific proteins by Peletier and Hall (1971).

The genetic analysis of differences in pathogenicity has begun (McGeary, 1980). Only limited progress has been achieved because of the necessarily selective techniques employed. Recent work by Clarkson and Heale (unpublished) reveals that certain diauxotrophic mutants of *V. albo-atrum* are able to colonise hop effectively (and antirrhinum) and are being exploited to search for heterozygous diploids and recombinants in these hosts. Extensive genetical studies on *V. tricorpus* and *V. dahliae* attacking cotton in the USSR has been carried out by Kasyanenko *et al.* (1978) and by Tolmsoff (1973) in the USA. Alternative techniques for studying the inheritance of natural variation in pathogenicity will also be considered.

Strain Specialisation, Genetic Compatibility and Colonisation Studies in Verticillium Wilts of Lucerne and Hop

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Isolates of *Verticillium albo-atrum* Reinke et Berth. from wilting lucerne *(Medicago sativa)* in the U.K. and France, when tested under standard conditions (temp. 12-21°C), showed significant differences in virulence and the extent of colonisation in cultivars of this host possessing different levels of resistance. Certain North American isolates of the lucerne strain (currently spreading in the U.S.A. and Canada) caused increased wilt over a higher temperature range (17-30°C) than the European isolates tested, indicating a possible geographical specialisation of the pathogen under conditions of relatively high soil/field temperatures during the growing season under American, as compared with European, conditions.

Isolates of *V. albo-atrum* attacking hop *(Humulus lupulus)* fall into two broad groups of strains: (1) *fluctuating,* usually causing mild symptoms in isolated plants and of little economic importance; (2) *progressive,* causing defoliation and death of hop, resulting in a serious disease problem in the U.K. Over the past 10 years, new strains of the fungus have evolved, capable of causing 'progressive wilt' in hitherto resistant hop cultivars. Preliminary genetic studies aimed at investigating the possible role of the parasexual cycle in the evolution of those new strains, indicate a degree of compatibility between fluctuating and progressive isolates, suggesting that gene-flow between (and within) such populations might occur at some stage during the saprophytic or parasitic phases in the pathogen life cycle. Forced heterokaryons between complementary, diauxotrophic parents, on minimal medium, often gave rise to unstable diploid prototrophic sectors. The pleiotropic effects of auxotrophic marker genes, as well as mutations affecting colour and morphology, on the expression of virulence in hop will be discussed, as well as the different rates of colonisation of wild-type fluctuating and progressive strains.

Integrated Control of Verticillium dahliae in Potato

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Under conditions of high evapotranspiration, as found in the Negev Region of Israel, Verticillium wilt can be an extremely damaging disease. The use of an integrated control approach has proven to be extremely successful in controlling the disease. The major points in the methology used are as follows:

1) Developing a critical method for measuring the tolerance or resistance of potato cultivars.
2) Determining what other biotic factors, if any, are operative in the disease situation.
3) Developing economic methods of chemical control as an adjunct to tolerant cultivars.

In brief, the following should be noted:

1) Assessment of tolerance was carried out by growing cultivars under field conditions, in infested and non-infested soil.
2) The free living nematode, *Pratylenchus thornei,* was found to act as a predisposing agent to early infection by the fungus.
3) A cheap, efficient method of metham-sodium application was developed to control the nematode. This method is based on application of the biocide via the sprinkler irrigation system. Two hundred and fifty liters/hectare are used for this purpose.

The results of the above research have been adopted by growers with concomitant yield increases of up to 75%.

Studies on the Chemical and Biological Control of Verticillium Wilt Disease

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Studies on the chemical and biological control of Verticillium wilt disease *(Verticillium dahliae)* have been conducted. In *in vitro* experiments the fungicides Benlate, Derosal, Pomarsol Forte and Brassicol 75 WP (PCNB) and the fungus of *Trichoderma viride* were investigated. Of these chemicals Benlate and Derosal were best in controlling the fungus. Pomarsol Forte was effective on mycelial growth and sporulation, suppressing microsclerotial formation. Brassicol 75 WP was not effective on mycelial growth, sporulation or on the formation of microsclerotia. *T. viride* was hyperparasite on *V. dahliae.*

In *in vivo* conditions Benlate, Pomarsol Forte, Ceresan P (PCNB) chemicals and the fungus *T. viride* were used against the Verticillium wilt on cotton, okra and bean plants. These experiments are still in progress.

Pathogenicity Variations in One Tomato Isolate of Verticillium dahliae

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The pathogenicity of a tomato isolate of *Verticillium dahliae* was studied using cultures of different ages.

Three-week-old tomato plants (variety Marmande) were inoculated by soaking in a suspension of conidia (10^6 sp./ml), then sprayed, after planting in sand, with the same spore suspension. Inoculated plants, compared with the controls, were stunted, with localised yellow or wilted areas in the foliar system.

Cultures obtained from a single monosporous culture (initial clone) were inoculated after incubation periods of 15 days, 1, 2, 3 and 4 months. Pathogenicity estimated from the growth of the first internode or epicotyl was significantly higher in the 2-, 3- and 4- month old cultures than in the 15 day and 1- month old cultures.

The pathogenicity of sub-clones (each obtained from a single conidium) was variable when conidia were harvested from 3- and 4- month old thalli: it could be equal, higher or lower than the pathogenicity of the initial clone.

The increase in pathogenicity in older cultures could be explained by the formation of variants more pathogenic than the initial clone. These variants would be automatically at an advantage after inoculation. These results seem to indicate the importance of the saprophytic phase of *Verticillium* in the subsequent pathogenic behaviour of the fungus.

Biotic and Abiotic Elicitation of Terpenoid Aldehyde Phytoalexins in Cotton

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The Verticillium-resistant cotton cultivar Seabrook Sea Island *(Gossypium barbadense)* infected with *Verticillium dahliae* showed localization of five terpenoid aldehyde phytoalexins, detected histochemically, in isolated paravascular parenchyma cells of stems 2 days after inoculation. Both discrete localization and diffuse deposition of phytoalexins occurred during a 2 week infection period. The diffuse deposits appear to arise by diffusion from initial, localized sites. Infiltration of stem segments with 10^{-3} M $CuCl_2$ also induced similarly localized deposits of the terpenoid aldehydes in paravascular parenchyma cells 3 days after infiltration. Extracts of stele tissue from Verticillium-inoculated and $CuCl_2$-infiltrated stems both revealed the presence of the same five terpenoid aldehydes. Hemigossypol and 6-methoxyhemigossypol constituted 45 and 41 mole %, respectively, of the total terpenoid aldehyde in the inoculated stems and 80 and 17 mole %, respectively in the $CuCl_2$-infiltrated stems. Gossypol, 6-methoxygossypol, and 6,6'-dimethoxygossypol occurred as minor components in the extracts of both Verticillium-infected and $CuCl_2$-infiltrated stems. No terpenoid aldehydes were detected in the noninoculated and water-infiltrated stems. The histochemical data indicates that the paravascular cells responding to both *Verticillium* and $CuCl_2$ are biochemically specialized for terpenoid aldehyde synthesis and therefore are distinct from adjacent, morphologically similar cells. Furthermore, the elicitation of the same five terpenoid aldehydes by *Verticillium* and $CuCl_2$ indicates that both elicitors activate the same biosynthetic pathways.

Hybridisation of Verticillium albo-atrum Strains from Tomato and Lucerne

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Strains of *Verticillium albo-atrum* isolated from diseased plants of lucerne (L), potato (P) and tomato (T) were generally most virulent towards their original host species in subsequent pathogenicity tests. Lucerne strains (L) were most specialised in that only they caused disease of lucerne. P and T strains infected lucerne roots, but were never reisolated from the associated stem tissue.

Standard procedures for parasexual hybridisation using auxotrophic mutants to select heterokaryons and heterozygous diploids were used. Diploids were recovered with equal facility from complementary L x T and T x T strain combinations. The frequency with which heterozygous diploids were recovered seemed to be dictated mainly by the particular auxotrophic requirements, rather than the sources of the strains (lucerne or tomato).

The pathogenicity of auxotrophic mutants was generally significantly less than that of the wild-type prototrophs from which they were derived. Prototrophic haploids were therefore selected from heterozygous diploids for pathogenicity testing. Twenty-one independent prototrophic haploids from one particular L x T cross were tested for their capacities to cause disease in each potential host. Eleven of these were recombinants capable of causing severe disease in both lucerne and tomato. That and other aspects of the results suggest that the different pathogenicities of lucerne and tomato strains are not caused by one gene.

Effect of Plant Age on the Susceptibility of the Peanut cv Tamnut to Verticillium Wilt

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Plants of the peanut cultivar Tamnut were inoculated with *Verticillium dahliae* at various ages (4, 6, 8 and 12 wk). Inoculations were accomplished by soaking the roots for 45 min. in a conidial suspension containing 1×10^6 conidia/ml. Roots of non-inoculated control plants were soaked in distilled water for 45 min. Plants were transplanted in pots (16.5 cm) containing a mixture of soil, fine shredded peat and sand (4:1:5;v/v/v). Plants were maintained under greenhouse conditions favurable to the growth of peanuts.

Verticillium wilt symptoms appeared on all inoculated plants within two to three weeks after inoculation. Early symptoms consisted of epinasty, marginal and general chlorosis of leaves, and short internodes. Later developing symptoms involved flaccidity and defoliation of leaves and wilting of plants. Control plants consistently showed no disease symptoms for the duration of the experiment.

Sixty days after each of the inoculations, the effects of infection on plants were evaluated. At that time, aerial parts and roots were separated. Total pegs and pegs with fruits, per plant were determined. Aerial parts and roots were dried for 15 min. in a microwave oven at maximum power and dry weights were recorded.

Reductions in root mass, aerial plant parts, total pegs, and pegs with fruits were noted on all inoculated plants as compared to non-inoculated controls; however, the reductions were less drastic in plants inoculated at eight or 12 weeks of age.

The Elicitation of Lucerne (Alfalfa-Medicago sativa L.) Phytoalexins by Verticillium spp.

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Only strains of *Verticillium albo-atrum* (or *V. dahliae)* isolated from lucerne (alfalfa - *Medicago sativa* L.) are capable of causing a wilt disease in this plant; all other strains of these fungal species are non-pathogenic. Lucerne produces phytoalexins in response to infection and a close correlation exists between the virulence of the fungal strains and the amount of phytoalexin produced viz. the non-pathogenic strains induce considerably more of these antifungal compounds than do the pathogenic strains. Similarly, the non-pathogenic strains are more sensitive to the inhibitory effects of the phytoalexin than are the pathogenic strains. A fraction has been isolated from the culture filtrates of the fungus which is capable of eliciting phytoalexin production. The purification and properties of the compound(s) involved will be discussed.

Behaviour of the Cells of the Xylem Parenchyme After a Vascular Stress

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The structural evolution of the cells lining the vessels was camparatively studied in carnation after injection of spores either of *Phialophora cinerescens* (a parasite specific to carnation) or *Verticillium dahliae* (non-pathogenic to carnation) and on sham-stressed plants.

Fixations for electron microscopy were carried out 6h, 12h, 24h, 48h, 3 d, 4 d, 5 d, 6 d, 12 d and 18 days after the stress. Pictures of the ultrathin slices were obtained at low magnification. One thousand of these pictures were examined after enlargement. Only the cells which were obviously lining the vessels were used in the observation. They were ranged in classes while their total and mitochondrial areas were measured. Several criteria were used for characterization of the vessels (type, state of the walls) and of the vascular gums (occurrence, abundance, structural aspect). The data were computed with the aid of microprocessors.

The results of this work confirm and precise the fundamental role of vessels-associated cells in the genesis of vascular gums. Two types of cells (called C_1 and C_2) with different structures naturally line the vessels (70% C_1, 30% C_2). A few hours after introduction of the vascular parasite, the two cellular types differ in reacting and undergo dramatic changes (10 types of change were recognised). A large increase in mitochondrial areas (up to 25% of the total area in cells derived from type C_1) implied an intensive increase of the metabolic activity. Secretion systems progressively appeared and were related with the abundance and the type of gums produced into the vessels.

In conclusion, in the caulinary xylem of carnation, two types of cells show different functions (at least in part) triggered by a vascular stress. The response of these cells differs depending on the severity of the stress (slight and delayed response to a sham-stress, large and early response to a severe parasitic stress followed by death only with the pathogen).

Verticillium dahliae **Kleb. in Iran**

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Verticillium dahliae Kleb. has been recognized in Iran since 1953. Damage to crops by *Verticillium,* especially in the northern part of Iran, has been very high. In Mazandaran up to 50% of cotton has been damaged. Cotton variety Coker 100 wilt and Acala 1517 C from the U.S.A. and 108 F from the U.S.S.R. and others were sensitive to the pathogen. Only one line, no. 349 from New Mexico (1955), was found much more tolerant to Verticillium wilt.

This line was very late maturing and when planted in non-infested soils yielded less than Coker 100 wilt by 20-30%. The studies confirmed that line no. 349 was very much more tolerant and was a good source of resistance. The hybrid of Coker 100 wilt x 349 was oustanding. Its yield was high with better ginning percentage and with very good tolerance. It showed its superiority to all other hybrids in wilt-infested regions.

In 1967 this hybrid was named 'Sahel'.

A Study of the Verticillium dahliae Kleb. Ecology in Soil with the Use of Membrane Chamber Techniques

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For ecological studies of *Verticillium dahliae* in soil combined membrane chambers were used. An inoculum of the fungus was placed on a membrane filtre, wrapped into a fine-fibre glass material and inserted into a joint slide frame. The chamber thus mounted was installed into soil. After the incubation period the chamber was taken out, treated as described and examined microscopically.

It was demonstrated that *V. dahliae* microsclerotia germinated in soil. The germination was considerably poorer than in a humid chamber; the hyphae, formed occasionally, gave rise to new microsclerotia which differed from the initial ones (obtained in the laboratory) by their smaller size and regular shape. They did not germinate in soil.

The membrane chamber technique was also employed in a study of antagonistic (competitive) relationship of micro-organism in soil. To this end a second (saprophytic) fungus was mounted into the chamber together with *V. dahliae*. Their relationship and development were followed as described above. An antagonistic fungus *Gliocladium,* which proved to be effective for cotton wilt, was extracted.

The use of the membrane chamber, together with immuno-fluorescent staining techniques, enabled distinction to be made between mycelia of *V. dahliae* and that of other fungi. Details of the procedure are described.

Possible Origin and Participation of Toxin in Verticillium Wilt of Tomato

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The host-parasite interactions resulting in pathogenesis in Verticillium wilts are very complex, apparently involving enzymes, toxins and plants hormones. While investigating the physiology of pathogenesis in Verticillium wilt of tomato, Pegg and Cronshaw (Physiological Plant Pathology 8:279-295; 9:33-44) demonstrated the presence of two toxic fractions in Verticillium culture fluids and observed a toxic synergism between ethylene and these toxic fractions.

We have confirmed Pegg and Cronshaw's observations on the presence of two toxic fractions in Verticillium culture fluids, and have demonstrated that the proteinaceous toxin is in fact, endopolygalacturonase (endoPG). A synergism between the endoPG and the carbohydrate toxin generated by the fungus has also been observed. The carbohydrate alone is not toxic, however it is extremely toxic to tomato cuttings that have previously been treated with purified endoPG. We can obtain a carbohydrate of similar molecular weight and synergistic specificity by two other techniques. Acid hydrolysis of Verticillium mycelium yields a carbohydrate fraction which is toxic to tomatoes only after they have been treated with endoPG; and a fraction with similar characteristics can be obtained by digestion of Verticillium mycelium with an enzyme preparation obtained from tomato cell walls. The mycelium-degrading enzymes from tomato cell walls are readily solubilized from these walls by purified endoPG.

The above information, and observations on the relative sensitivity of several tomato isolines to these toxins will be presented within the framework of a generalized model for the physiological events which result in pathogenesis in Verticillium wilt of tomato.

The Role of Toxins in the Pathogenesis of Verticillium dahliae on Potato

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Two isolates of *Verticillium dahliae,* one pathogenic to potato and the other non-pathogenic, were grown in an aqueous synthetic medium. Two major protein-lipo-polysaccharides (PLP) were isolated from the culture filtrate. The materials produced by the pathogenic isolate were toxic to potato leaflets in a laboratory bioassay while the materials from the non-pathogenic isolate were not toxic. There is however a serological and molecular weight similarity between the two major products of the two isolates.

In developing a bioassay for *V. dahliae* under controlled conditions, the PLP from the non-pathogenic isolate was used as a negative control since it was not possible to inactivate the toxins and retain their molecular configuration.

Antisera against the major peak reacted in an immunodiffusion test with an extract from Verticillium infected potato tissue.

The Impact of Verticillium Diseases in Agriculture

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Five species of *Verticillium* are currently recognized as plant pathogens, but of these *V. albo-atrum* and *V. dahliae* are of greatest importance. Diseases caused by these two species are widespread throughout temperate regions of the world, Europe, North America, U.S.S.R. and Australasia, and in the case of *V. dahliae* in subtropical and tropical environments, for example India, South America and Africa.

Losses of crop plants (field, plantation and glasshouse) on a world scale are considerable, with the prospect of increasing severity. A major concern is the occurrence of *Verticillium* in previously uncropped soils or on land not previously cropped with susceptible species. The importance of weed and non-host crops as sources of pathogenic Verticillia in agricultural systems and induced pathogenicity of soilborne species is discussed. The uncontrolled dissemination of infective vegetative and seed plant material within and between countries as a source of increased infection is considered.

In spite of intensive research on wilt diseases during the last fifty years, the agricultural trend towards monocultures and the narrowing of the host genotype has led to increased world-wide epidemics. Notwithstanding the considerable ability of the pathogen to overcome genetic and chemical control systems, agricultural production in wilt areas in future must increasingly rely on plant breeding integrated with chemical control.

Screening for Resistance Against Verticillium dahliae in Avocado Rootstocks

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Increased avocado growing in Israel was accompanied by a considerable increase in Verticillium wilt in the new plantations. The use of cultivated land that previously grew vegetables intensively is partially responsible for this increase. However, diseased trees quite often appeared on land used for citrus production for many years. The distribution of infected trees in certain groves was associated with certain rootstocks which suggested differences in susceptibility among avocado rootstocks. *Verticillium dahliae* can penetrate unwounded roots of avocado seedlings. The fungus reaches the vascular system and newly developed spores are translocated acropetally in the xylem. Four days after inoculation the fungus could be detected in the top of the plant - 60 cm above inoculation site. Inoculation of seedlings often resulted in symptomless plants, most of which contained viable propagules of the fungus at various points along the stem. This type of resistant reaction was of no practical value since the fungus could be translocated into the scion, and later incite disease symptoms. To separate resistant rootstocks from symptomless carriers, a simple technique for stimulating symptoms appearance was developed. A reliable and fast screening procedure was developed to locate resistant individuals in seedlings populations.

Effects on Membrane Permeability of Two Glycopetides Produced by Phialophora Cinerescens (Wr.) Van Beyma

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Phialophora cinerescens inhibited the growth of cultivated carnation plants *(Dianthus caryophyllus* L.). The culture filtrates of the fungus were shown to contain two low molecular weight glycopeptides - one neutral, the other acidic - which disturbed the development of carnation seedlings. Both these compounds slowed down the root elongation. Only the neutral one reduced hypocotyl growth. The two glycopeptides were isolated from the stem of infected carnation plants. The acidic one was uniformly distributed in the stem, the neutral one was more abundant in the upper part where growth inhibition was observed. The close correlation between cell enlargement and membrane permeability led to the study of the effects of the glycopeptides on transport processes. We also investigated whether glycopeptides might antagonize the capability of fusicoccin to stimulate electrogenic H^+ extrusion. The neutral glycopeptide inhibited the acidification of the incubation medium by *Acer pseudoplatanus* cells. It decreased the H^+ extrusion and the uptake of $86_{RB}{}^+$. These effects became much more evident when H^+ extrusion and $86_{RB}{}^+$ putake were stimulated by fusicoccin. In our experiments, the acidic glycopeptide did not disturb the activities of the cell membrane.

In infected carnation plants, such modifications of the membrane permeability induced by the glycopetide could lead to growth inhibition.

Further Studies on Heterokaryon-Compatible Populations Within Verticillium dahliae

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In an earlier published report (Phytopathology *69:* 1186-1189. 1979) 19 isolates of *Verticillium dahliae* were tested for their ability to form heterokoryons with each other. Albino and brown microsclerotial mutants were paired, and heterokaryosis was indicated by a line of black microsclerotia where the two mutants touched. Four populations were thereby defined; isolates from the same population formed heterokaryons with each other, isolates from different populations did not.

This earlier study has now been extended to 94 isolates of *V. dahliae* and 86 of these have been assigned to 16 different populations, labelled P1 through P16, on the basis of heterokaryon-forming ability. Limited data suggest the isolates within a population may be very similar genetically and may, therefore, have limited virulence and host range. As an example, all 16 of the so-called defoliating isolates of *V. dahliae* in this sample belong to P1. Four of 6 pepper isolates were relegated to P5, and 7 of 9 tomato isolates fell into P2. Some populations show a restricted geographical distribution; e.g., all or nearly all isolates of P1 and P4 were found only in the western hemisphere.

Of the 8 remaining isolates in this sample two may be diploid. The other 6 isolates failed to form heterokaryons even with themselves.

Although we know the host plant from which each of the 94 isolates was obtained, we have made only limited tests to determine the extent of their host range and virulence. More pathogenicity tests are needed before any conclusions can be made about the significance of these heterokaryon-compatible populations in the epidemiology and evolution of the species *V. dahliae.*

The Range of Foliar Symptom Expression in Verticillium-Infected Hosts: an Ultrastructure Survey

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Fungi of the genus *Verticillium* cause vascular « wilt » disease in a large number of plants. In some cases the first foliar symptom is a slight flaccidity at the tip of the leaf lobes, followed by more extensive wilting, and, eventually, chlorosis and necrosis. This is the classical « wilt » syndrome. In many hosts, however, chlorosis and necrosis occur rapidly and the leaves either dry up or dehisce with very little actual wilting response. Detailed ultrastructural analyses of the leaves of « wilting » (chrysanthemum) and « non-wilting » (sunflower) hosts infected with the same fungal strain have indicated certain cellular differences in host responses; these include quantitative and temporal differences involving the formation of smooth and bubbly coating materials, plugs, and tyloses in the xylem vessels; as well as the amount of vascular browning (i.e. phenolic deposition) and the formation of the necrotic band in and around the vascular column. Every host-parasite system is, to some extent, unique; therefore, the question arises whether any of the characteristics observed in chysanthemum and sunflower bear a consistant relationship to the type of symptom expression. An ultrastructure survey (SEM, TEM) of leaves of ten differents hosts infected with *V. dahliae* or *V. albo-atrum* show that « wilting » hosts such as snapdragon and eggplant have large amounts of smooth and bubbly coating materials in their vessels and also an increase in other forms of vessel blockage (i.e. plugs, tyloses) but the latter is less consistent. « Non-wilting » hosts such as maple or hops (acute tiger-stripe syndrome) have virtually no smooth coating material and no plugging, but a higher proportion of fibrillar coating in the vessels and greater vascular browning. The necrotic band is restricted to intermediate hosts such as potato. The cytological characteristics vary in a continuous way, consistent with visual foliar symptoms, suggesting some fundamental relationship between the two.

The Effect of Daylength on Verticillium Wilt of Sunflower

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Sunflower inbred lines CM162 (susceptible to Verticillium wilt) and CM144 (resistant) were grown under combinations of short (10 hours) and long (16 hours) daylengths before and after inoculation at the 4 or 8 leaf stage with *Verticillium dahliae* isolates VM or VNur. Both lines flowered earlier under short daylength.

Symptoms appeared sooner on both lines inoculated with VNur than with VM, sooner on CM162 than on CM144, and were more severe as well as appearing sooner under short than long days.

VM induced much more severe symptoms than VNur on CM162; both isolates induced only slight symptoms on CM144. Leaves formed after initial symptom development on CM144 appeared healthy. Similar « recovery » occurred on CM162 inoculated with VNur but not with VM. « Recovery » of *Verticillium*-inoculated sunflowers had not occurred in our previous work.

Inoculation of CM162 with VM gave 60% of plants producing infected seed, and 30% of seed infected; inoculation with VNur gave 25% of plants with infected seed, and 7% of seed infected. Inoculation of CM144 with VM gave 5% of plants with infected seed, and 0.9% of seed infected; inoculation with VNur gave 9% of plants with infected seed and 1% of seed infected.

The effect of daylength on symptom severity appeared related not to induction of flowering but to vigor; uninoculated plants under long days were more vigorous, and inoculated plants had more severe symptoms, than plants under short days. The effect of daylength on seed infection was not consistent.

Possible Causes and Sites of Vascular Occlusion in Tomato Infected with Verticillium albo-atrum

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Various evidence implicates increased resistance of xylem vessels to water flow as the major cause of water stress in tomato plants infected with *Verticillium albo-atrum*. The small independent vessels of petioles and especially leaves are particularly vulnerable to occlusion during pathogenesis (c.f. large network bundles in stems), but a critical study of the influence of the pathogen and its products on this tissue is lacking.

Such effects could result from the presence of high molecular weight polymers in vessels; these can derive from the pathogen or may result from enzymic degradation of xylem wall polygalacturonide at pit membranes and perforation plates. The latter possibility is suggested by the appearance of gels and high pectin lyase activity in vessels before appearance of symptoms.

The relative effects on flow rates through xylem of stems, petioles, leaf midrib and minor veins, of a range of dextrans, polyethylene glycols and polygalacturonides of known sizes were determined with a modified Scholander pressure bomb at *ca* 0.3 bars. Vascular flow was measured electronically as rate of drop formation from severed ends of large bundles or from minor veins by gravimetric techniques. The resulting reductions in flow are compared with those induced by high molecular weight polymers and purified polygalacturonide hydrolase and lyase from cultures of *V. albo-atrum,* and with the conductivity of xylem in infected tissue.

The sites of occlusion in leaves were determined by microscopic examination of the distribution of fluorescent dye (calcofluor) supplied after passive uptake of the various compounds. Blockage often occurred at independent xylem vessels (minor viens) which are compared ultrastructurally with similar vessels in infected plants.

Chemical Control of Verticillium wilts

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Control of any soil-borne vascular disease poses the critical logistic problem of placing the controlling agent (whether chemical or biological) at the appropriate time and in sufficient quantity at a location where it can be effective against the pathogen, with minimal adverse effect on the environment, and at an economic cost. The site of action may be in the soil, at the soil/root interface or in the xylem of the host plant.

Chemical control in the soil (by direct fungicidal or fungistatic action, or indirectly through effects on the microbiota) requires dispersal of the controlling agent through a large volume of soil, usually entailing diffusion of the chemical in the liquid or gaseous phase from the applied liquid, powdered or granular formulation. The viable inoculum is likely to be such a small fraction of the total soil volume that most of the chemical can have no direct action on the pathogen, although it may incidentally control weeds, nematodes etc. Improvements in techniques for incorporating chemicals into the soil do not overcome problems associated with deep-rooted crops (e.g. hops) and 'difficult' soils (e.g. heavy clays).

Chemical control in the host-plant (by direct fungicidal or fungistatic action, or indirectly through effects on host structure, physiology or response to infection) requires systemic action. Acropetally-directed apoplasmic translocation from soil drenches constantly removes the fungicides (e.g. benzimidazoles and thiophanates) away from the preferred site of action viz. the root system. Future chemical control will increasingly utilise downward-moving (symplasmic) materials, probably applied to leaves and shoots by electrostatic spraying. Modes of action may include: fungicidal action within the plant and (or) after diffusion into the rhizosphere; modification of host responses to enhance resistance; or modification in quantity or quality of root exudates to alter the activity or constitution of the root-surface microflora. Application of the disease-controlling agent via the host plant enables it to be placed at the most relevant locations, especially at or near the initial points of contact between host and pathogen.

Occurrence of Verticillium Wilt in Weeds in Olive Orchards

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Olive is one of the main crops in Greece. Verticillium wilt has recently become the main disease problem, as described in earlier papers, at least on var. « Konservolia ». The disease is more severe in fields where *Verticillium dahliae* propagules are abundant in the soil, particularly in orchards intercropped with vegetables, cotton or other Verticillium susceptible hosts. In such intercropped fields the perpetuation of the fungus is facilitated from season to season on plant debris and its inoculum potential is also increased. In many cases, however, the fungus has been recovered from diseased trees growing in fields never previously planted to sensitive crops nor irrigated with water flowing through such crops. The involvement of weeds is suspected to play an important role in the epidemiology of Verticillium wilt in olive, as has been well documented in other crops.

In an extensive survey of the weeds growing in olive orchards it was found that many harbored the fungus either as a susceptible host or as symptomless carriers. Isolations were attempted from 64 weed species belonging to 28 families. The fungus has been isolated from the vascular tissue of the following species: *Amaranthus retroflexus, Xanthium spinosum, X. strumarium, Solanum nigrum, Capsella bursa-pastoris, Malva sylvestris, Chenopodium album, Geranium dissectum, Senecio vulgaris, Calendula arvensis, Avena sativa, Avena fatua.* The reported weeds except *A. retroflexus* are new host records of *V. dahliae* for Greece. The last three species have not been traced as hosts in the searched literature. *Xanthium* spp appear to be very susceptible hosts. The above data indicate, therefore, that weeds growing in olive orchards might be of major importance for the perpetuation of the fungus and disease incidence, increasing also inoculum level.

Race 2 and Defoliating T-1 Strain of Verticillium dahliae in Greece and Other Mediterranean Countries

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Race 2 of *Verticillium dahliae* has been frequently reported in the United States since 1962. Although this particular race, which overcomes the resistance of tomato varieties possessing the *Ve* gene, is widespread in the States, it seems to be extremely rare in the Mediterranean region regardless of its record in South France, Italy and Greece. Early tests in Italy showed the occurrence of race 2, isolated from a melon plant, while more recent findings in Greece referred to an eggplant isolate pathogenic to resistant tomato varieties. These two isolates are the only reports of race 2 in both countries. Katan in Israel has tried to isolate race 2 but to date without any success.

A severe defoliating strain in *V. dahliae* (designated T-1 by Schnathorst) has not been found in Greece. This was demonstrated by inoculation of cotton cultivars with a large collection of Greek cotton *V. dahliae* isolates under greenhouse conditions. It was also based on the morphology of microsclerotia (lack of linear microsclerotia on water agar) and on the absence of anastomoses with a T-1 isolate obtained from Davis, California. Occurrence of T-1 strain has not been reported in other cotton growing countries of the Mediterranean sea (Turkey, Syria, Israel, Egypt).

The evidence presented so far indicates that the pathogenicity of *V. dahliae* isolates in the Mediterranean countries differs from that of the American continent. This could be attributed to the following reasons: 1) differences in population synthesis of the fungus between America and Mediterranean countries; 2) limited selection pressure imposed by the cultivation of Verticillium resistant tomatoes or Verticillium tolerant cotton varieties.

Our results suggest the increased use of Verticillium-resistant tomatoes and encourage tests of wilt tolerant cotton cultivars under Greek conditions with the aim to restrict extensive annual losses inflicted by the pathogen.

Failure of Two Growth Retardants to Suppress Verticillium Wilt Symptoms and Increase Yield in Cotton Field Trials in Greece

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Verticillium wilt constitutes the most severe disease of cotton in Greece. Testing the effect of growth regulators to control the disease is one of the modern approaches to the control of the problem. Our aim was to investigate whether these non-fungitoxic compounds can reduce disease symptoms and increase yield under field conditions in Greece.

Three-year field trials were carried out in Boeotia and Pthiotida in central Greece, where the early maturing and very productive variety 4-S is almost exclusively cultivated. The growth retardant chemicals Pix and Cycocel were used at the initial flowering stage (first week of July) at the doses of 25 or 60 g a.i. per ha. Two kinds of experiments were conducted in naturally-infested soils by foliar applications. In one of the experiments the plots covered an area of half an acre each while in a second procedure experimental plots of 40 m^2 each were used.

Although there was a definite reduction in plant height, neither symptom mitigation nor increased yield could be achieved by the foliar application of these compounds. This was shown by a comparison of the mean disease indices and total cotton seed production of the treated plots.

Growth retardants failed to achieve any control of Verticillium wilt of cotton in central Greece. This result, however, may be different in northern regions where cotton matures late in autumn.

A Cytoplasmic System of Inheritance Affecting the Variability and Differentiation of Verticillium

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Acriflavine, acridine orange and ethidium bromide were found to have a similar effect on *Verticillium,* increasing significantly the spontaneous rate of production of stable hyaline (Hyl) and partially hyaline (Hyl^+ . p) variants. They were also found to have a more or less specific effect in inducing mitochondrial mutations (i.e. mutations concerning the respiratory system of the fungus, mitochondrial structural proteins and mitochondrial protein synthesis) when compared with mutations arising from U.V.-irradiation and/or NTG treatment. The mode of inheritance of most of these morphological and mitochondrial mutations was followed by employing heterokaryon tests and genetic analyses via the parasexual cycle. With the exception of two mitochondrial mutations (one confering resistance to antimycin A and another resistant to cyanide) which were found to be nuclearly controlled, all the rest were found to have a cytoplasmic pattern of inheritance. *In vivo* mitochondrial preparations from a darkly pigmented (Hyl^+), arginine requiring, amytal sensitive strain (Hyl^+ arg amy) as well as from a hyaline, arginine requiring, amytal sensitive strain (Hyl arg amy) were microinjected by means of micromanipulation into a hyaline, adenine requiring, amytal resistant strain (Hyl ade amy^r) and a darkly pigmented, leucine requiring, amytal resistant strain respectively. The results obtained from these microinjections, suggested that the Hyl^+ and amy^r markers were somehow linked. Moreover, the isolation of recombinant types (one Hyl ade amy^r in the first case, and one Hyl^+ leu amy^r strain in the second case) indicates the possibility of mitochondrial DNA recombination occurring in nature.

Relationship of Inoculum of *Verticillium dahliae* to Disease in Tomato Plants

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Tomato (*Lycopersicon esculentum* Mill.) seedlings were inoculated by dipping roots in suspensions of conidia of *Verticillium dahliae* Kleb. There was a linear relationship between inoculum concentration and infection at 21 days after inoculation in steam-disinfested soil. Thereafter the number of infections per plant increased. At higher concentrations data indicated a synergistic interaction between conidia. Synergism was more pronounced and was detected earlier in soil not disinfested before inoculation. When conidia from 3-, 7-. and 14-day-old cultures were used, the greatest response was from 3-day-old cultures. A lower total response and lower rate of response to older inoculum indicated a decrease in aggressiveness of conidia with age. Older plants were less affected by the pathogen when plants inoculated at the fourth and sixth leaf stages with minimum root disturbances were compared. There was also an increase in infection with an increase in the volume of root zone infested. In field experiments infection reduced stand when roots were disturbed at inoculation. With minimum root disturbance yields were reduced without a reduction in stand.

Studies on Germination of Spores of Verticillium albo-atrum

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Experiments on germination of spores of *Verticillium albo-atrum* either in or on water drops showed that (a) in ordinary drops on glass slides germination was greatest at the margins, (b) with water drops formed on wax, greatest germination occurred at the margin and on the surface of the hemispherical drops, and (c) for drops with high surface tension conidiophores and conidia were produced on the surface in 3 days. These results may have implications in relation to infection and build-up of secondary inoculum under field conditions.

Spores of *V. albo-atrum* and *Aspergillus niger,* singly and in combination, were sown in hanging drops in Van Tiegham cells to investigate their interaction in relation to germination at extreme temperatures for growth (15C and 30C). At 15C spores of *V. albo-atrum* alone germinated with up to 50% of the spores producing germ tubes up to 100 µm long. Spores of *A. niger* alone did not germinate. In combination *A. niger* did not germinate and germination of *V. albo-atrum* was either completely inhibited or what germ tubes did grow were stunted. At 35C *V. albo-atrum* alone did not germinate while *A. niger* germinated up to 50% of its spores, profuse mycelium grew, and conidial heads began to form at 72 hr. In combination *V. albo-atrum* did not germinate. *A. niger* performed about the same as alone until 48 hr when many conidial heads were formed, and 'kinky' hyphae made the mycelium appear different from that of the fungus growing alone. This experiment shows that at temperature thresholds for growth of these two fungi, each may influence growth of the other which has not yet reached its threshold. This may have implications for their survival under stress.

A New Variety of Verticillium sphaerosporum, an Endoparasite of Nematodes and Antagonist of Soilborne Plant Pathogens

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An isolate of *Verticillium* sp. was obtained from conidia produced on dead nematodes on water agar originally plated with decaying strawberry root tissues. This fungus showed endoparasitic effects against some nematodes tested, including *Aphelenchoides, Cephalobus,* and *Panagrolaimus* sp. It is morphologically similar to *Verticillium sphaerosporum* Goodey, except for the formation of cylindrical conidia (9.3 μm x 2.3 μm), but was identified as *V. sphaerosporum* var. *bispora* in this study. In dual culture on potato-dextrose agar with each of 90 isolates of various fungi including several soilborne plant pathogens, it caused more than 50% inhibition of radial mycelial growth rates in *Alternaria* sp., *Phoma* sp., *Pithomyces* sp., *Pythium splendens, P. sylvaticum, Rhizoctonia solani,* and *Trichocladium* sp.

Sources and Genetics of Verticillium Wilt Resistance in Major Crops

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Verticillium wilt limits production of field and fiber, vegetable and oil, beverage and herb, and fruit and nut crops throughout the temperate world. Major crops affected are alfalfa (lucerne), lupine, clover, tobacco, cotton, hemp, tomato, potato, pepper, eggplant, cucumber, cruciferous vegetables, spinach, peanut, sunflower, safflower, coffee, cocoa, sage, rosemary, thyme, peppermint, stone fruits, strawberry, black-berries, avocado, mango, olive, and pistachio. Guayule, a potential rubber crop, and a number or ornamental crops are also susceptible. Sugar beet, castor bean, broad bean, onion, wheat, grapes, apples, pears, and quince have shown a tendency to susceptibility.

For many crops, resistant cultivars have lessened the impact of the disease. For others, problems remain which for the most part reflect the capacity of the wilt fungus to infect, and grow and sporulate within, vascular elements of resistant plants, and to invade their leaves. In some crops, for instance in cotton, wilt resistance appears to lack precise genetic control, partly because the expression of resistance in the young host is inadequate to prevent infection of tracheary elements. Once the pathogen has entered a vessel, its development depends upon structural and chemical features of that vessel and the composition of its aqueous contents. Qualities of these features, in turn, are modulated by host stress factors, and by growth rate and form of the host as determined by priorities of photosynthate partitioning, such as that for vegetative and reproductive uses. Perennial, wilt-susceptible hosts, such as olives, stone fruits, and wild cotton, may mimic resistance by recovery phenomena.

In the general absence of host immunity to infection by *Verticillium,* resistance may be measured by criteria such as the percentage of tracheary elements infected, the density of conidia in the vascular sap, the greatest vertical extent of infection, and the percentage of mature leaves infected. High productivity is not necessarily a valid gauge by which to measure resistance.

Occurrence of Verticillium spp. in Venezuela

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In Venezuela *Verticillium* spp. has been reported in several crops. However, its characterization and geographical distribution has not been studied yet. Up to the present time 22 random isolates of *Verticillium* spp. were obtained from potato *(Solanum tuberosum),* tomato *(Lycopersicon esculentum),* cotton *(Gossypium hirsutum),* cabbage *(Brassica oleracea)* and soil samples. Ten isolates were classified as *Verticillium dahliae,* four as *V. nigrescens,* four as *V. albo-atrum,* and one as *V. tricorpus;* three isolates from soil samples could not be classified. *V. dahliae* was isolated from potato, tomato, and cabbage; *V. albo-atrum* from potato and a soil sample, *V. nigrescens* from potato, tomato and cabbage; and *V. tricorpus* from potato. All isolates proved to be pathogenic to Kennebec and Sebago potato.

Some Aspects Concerning the Susceptibility and Tolerance of Hop Varieties Against Verticillium Wilt

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Verticillium wilt of hops is one of the most profit-limiting diseases in Bavarian hop growing areas. During the past few years a change in hop varieties has occurred from the original susceptible varieties to more tolerant ones. The quality and profitability of the tolerant varieties are lower, however, than those of the susceptible ones.

By examining susceptible and tolerant varieties at different dates, the rate of growth of the wilt fungus in the xylem vessels has been recorded. The isolation of *Verticillium* from young shoots was possible as early as the end of March in 1974 and was successful from tolerant varieties. This early isolation of the causative agent argues against reinfection from the soil each year. In contrast to 1974, the fungus was first isolated at the end of May in 1975 and was possible in only three cases from susceptible varieties. All examinations later in the year, as well as in subsequent years, yielded similar, variable results. Evidently climatic conditions, and subsequent differences in the growth of the host plant, play an important role in fungal migration from the hop roots into the shoots and further into the bines.

During the past few years we have tried artificial inoculation of the wilt fungus into susceptible and tolerant varieties. Inoculations took place from the beginning to mid July. The reisolation, done from the end of August to mid September, showed very restricted fungal growth in the tolerant variety Northern Brewer, in contrast to extensive growth of the fungus in the susceptible variety, Brewer's Gold. It is suggested that nonsusceptibility of hops is due not to difficult penetration of the parasite into the host, but rather probably to reduced fungal growth in the xylem due to different physiological conditions in the tolerant host plant.

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